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Pasteurization of chicken litter with steam and quicklime to reduce *Salmonella* Typhimurium

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Primary Audience: Live Production Personnel, Researchers, Veterinarians

SUMMARY

The nursery industry pasteurizes soil with steam and quicklime to reduce plant pathogens. The mechanism of action for quicklime is the resulting exothermic reaction that occurs when the chemical interacts with water and its ability to increase pH levels. These treatments may also reduce pathogens in a commercial poultry house. In this study, a steam sterilization cart simulated conditions used by the nursery industry to treat litter inoculated with *Salmonella enterica* serovar Typhimurium. A homogenized sample of litter was exposed to steam for 0, 5, 30, or 120 min. Quicklime was used at concentrations of 0 (control), 2.5, 5.0, or 10.0%. All steam treatments, with or without quicklime, significantly reduced *Salmonella* Typhimurium colonization by at least 3 orders of magnitude. Significant reductions were also observed in the treatments with quicklime alone. Both the steam and the quicklime treatments often reduced colonization to undetectable levels, even when samples were enriched. Therefore, we demonstrated 2 novel techniques for reducing *Salmonella* Typhimurium in poultry litter. Soil pasteurization potentially offers an environmentally sound means of reducing the pathogens present in used poultry litter.

Key words: pasteurization, litter, steam, quicklime, *Salmonella*

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DESCRIPTION OF PROBLEM

Poultry are typically reared on bedding material, such as wood shavings or rice hulls, to absorb moisture and facilitate the removal of feces at the end of the grow-out period. Removing these materials, disposing of them in an environmentally responsible manner, and replacing the bedding material can be costly. Because of these costs, multiple flocks of poultry are raised on the

same bedding substrate. Used litter may contain populations of pathogenic microorganisms that can infect current and subsequent flocks through coprophagy [1, 2]. Yeasts, molds, coronaviruses, rotaviruses, avian influenza viruses, *Staphylococcus*, *Pseudomonas*, *Escherichia*, *Campylobacter*, *Clostridium*, *Salmonella*, and *Eimeria* have all been isolated from used poultry litter [1, 3].

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Steam pasteurization is considered a safe and practical approach for reducing soil-borne pathogens in the commercial plant nursery industry. Steam has been used effectively to reduce brown root rot disease, *Deschampsia flexuosa* vegetation (weed), nematodes, grubs, and aphids since the 1800s [4–6]. It is possible that this method of pathogen reduction could be used in commercial poultry housing.

Luvisi et al. [7] reduced populations of 2 major fungi, which negatively affected plant quality, by applying steam and potassium hydroxide to produce an exothermic reaction. By a similar mechanism, calcium oxide, commonly referred to as quicklime (QL), may be used to enhance the effectiveness of steam pasteurization on used broiler litter. Water and QL react to form hydrated lime, carbon dioxide, and heat [8]. Lime is used to kill pathogens in sewage sludge before land application, and it is also applied to pastures that have an undesirably low pH [9, 10]. Bennett et al. [8] stated that manipulating the pH of litter may reduce the biological half-life of opportunistic and specific pathogens within poultry litter. We hypothesized that steam and QL would reduce *Salmonella enterica* serovar Typhimurium (ST) in broiler litter.

MATERIALS AND METHODS

Experimental Birds

Twenty-five market-aged straight-run Cobb × Ross broilers [11] were obtained from a poultry integrator and placed in a floor pen with fresh pine shavings. Birds were provided water and a balanced, unmedicated corn-soybean ration ad libitum that met or exceeded NRC [12] guidelines. Care was provided in accordance with a protocol approved by the Texas A&M University Institutional Animal Care and Use Committee.

Bacterial Culture

Salmonella Typhimurium was cultured in tryptic soy broth [13] for 8 h. The cells were washed 3 times with PBS (Butterfield's solution) by centrifugation ($2,000 \times g$) at 10°C , and the approximate concentration of the stock solution was determined spectrophotometrically (625 nm) [14]. The stock solution concentration

was confirmed by colony counts. Three replicate samples were serially diluted and spread plated onto brilliant green agar [15] plates containing $25 \mu\text{g/mL}$ of novobiocin [16].

Experimental Design

Three independent experiments were conducted to evaluate the effect of steam pasteurization and QL on ST recovery in broiler litter (Table 1). At placement, birds were orally gavaged with 3×10^8 cfu of ST/animal. Two weeks postchallenge, 100 lb (45.3 kg) of used litter was collected from the ST-challenged chickens. The litter was then mixed using a Wenger ribbon mixer [17] to ensure homogeneity. Twenty-pound (9.07-kg) aliquots were removed and mixed with 2 L of double-distilled water and the respective concentration of QL [18] to facilitate any chemical reactions. All treatments were mixed using a Hobart mixer [19]. The litter was then divided into two 10-lb (4.5-kg) portions and placed into a perforated nursery flat [20]. The perforated nursery flat measured 16.5 × 13 in. (41.91×33.0 cm), with a depth of 4 in. (10.1 cm) This depth would represent the built-up litter depth, depending on the number of flocks reared. Five plastic flats were then evenly distributed within a soil steamer cart [21] and steam [22] was applied. The remaining duplicate samples were housed inside a poultry barn with no steam. Six litter grab samples were collected for each treatment by using clean gloves and Whirl-Pak [23] filtered bags. Five grams from each litter grab sample was added to individual Whirl-Pak filtered bags containing 45 mL of Butterfield's solution and stomached [24] for 30 s. A series of 10-fold dilutions were then performed and spread plated onto brilliant green agar [15] containing $25 \mu\text{g/mL}$ of novobiocin [16] and incubated for 24 h at 37°C . One milliliter of each undiluted sample wash was also enriched with 9 mL of tetrathionate broth [25] and incubated for 24 h at 37°C . After this incubation period, 0.1 mL of each enrichment solution was spread plated onto brilliant green agar [15] and incubated for 24 h at 37°C . The limit of detection of the undiluted sample was 1×10^2 cfu/g of litter [26]. Dilution samples that had no bacterial growth but were positive after enrichment were assigned a value of $1.50 \log_{10}$ according to the

method of Corrier et al. [27]. Colony-forming units of ST were logarithmically transformed before analysis to achieve homogeneity of variance [28]. Data were analyzed by ANOVA using the GLM program of the SPSS statistical software package [29].

In experiment 1, treatments consisted of 1) no steam treatment and a control, 2.5, 5.0, or 10.0% QL, or 2) 120 min of steam treatment and a control, 2.5, 5.0, or 10.0% QL. In experiment 2, treatments consisted of 1) no steam treatment and a control, 2.5, 5.0, or 10.0% QL, or 2) 30 min of steam treatment and a control, 2.5, 5.0, or 10.0% QL. In experiment 3, treatments consisted of 1) no steam treatment and a control, 2.5, 5.0, or 10.0% QL, or 2) 5 min of steam treatment and a control, 2.5, 5.0, or 10.0% QL.

RESULTS AND DISCUSSION

This study was designed to evaluate the effect of steam and QL on ST populations in broiler litter. The authors found no research analyzing the effect of steam on poultry litter. However, steam treatments have been used in commercial plant nurseries to control microorganisms in an environmentally responsible manner for more than 200 yr [30]. Quicklime, when mixed with water, produces an exothermic reaction and may be used in combination with steam to elevate temperatures. In the current study, steaming of the poultry litter at the 2 longest time points and at each concentration of QL caused a significant reduction of ST (Figures 1 and 2). The 5-min steam treatment resulted in a reduction ($P \leq 0.05$) of ST to undetectable levels at the highest QL concentration of 10.0% (Figure 3). It is possible that the temperature and moisture associated with the steam treatment had an additive effect on QL activity (exothermic reaction and increased pH), causing the reduction in ST levels.

When the effect of QL without steam was evaluated in this study, ST levels were reduced ($P \leq 0.05$) in all experiments compared with the positive controls (Figures 1 to 3). We speculate that the reduction of *Salmonella* may be due to the increase in pH levels from these treatments. These results are similar to those reported by Bennett et al. [8], who observed significant reductions of *Salmonella* Enteritidis in less than

Table 1. Study experimental design

| Experiment | Steam, min | Quicklime, % |
|------------|------------|---------------|
| 1 | 0 | 0.0 (control) |
| | 0 | 2.5 |
| | 0 | 5.0 |
| | 0 | 10.0 |
| | 120 | 0.0 (control) |
| | 120 | 2.5 |
| | 120 | 5.0 |
| 2 | 120 | 10.0 |
| | 0 | 0.0 (control) |
| | 0 | 2.5 |
| | 0 | 5.0 |
| | 0 | 10.0 |
| | 30 | 0.0 (control) |
| | 30 | 2.5 |
| 3 | 30 | 5.0 |
| | 30 | 10.0 |
| | 0 | 0.0 (control) |
| | 0 | 2.5 |
| | 0 | 5.0 |
| | 0 | 10.0 |
| | 5 | 0.0 (control) |
| 5 | 2.5 | |
| | 5 | 5.0 |
| | 5 | 10.0 |

24 h after the litter application of 10 or 20% hydrated lime. These researchers also reported that hydrated lime, at concentrations of 5, 10, or 20%, increased the litter pH from 8.36 to 12.13, 12.53, and 12.57, respectively.

There is a need to identify and implement practical alternatives for treating litter to ease ongoing concerns associated with litter reuse and disposal, as well as the incidence of pathogens. Important factors that should be considered after litter treatment include the cost of the litter treatment, litter moisture, chemical properties, and the effect the treatment has on ammonia levels and bird performance in the subsequent flock. Locally available QL costs \$137.00/ton. Treating a broiler house containing 100 tons of litter with 2.5% QL would cost \$342.50 for the QL alone, not including the cost of the steam treatment. The steamed litter in this study was allocated into five 20-lb (9.07-kg) portions that were placed in a perforated nursery flat. The flats were then placed inside a 2-cubic-yard (1.53-cubic-meter) steamer cart and the litter was steam treated. Future studies should be conducted to evaluate the practicality of pasteur-

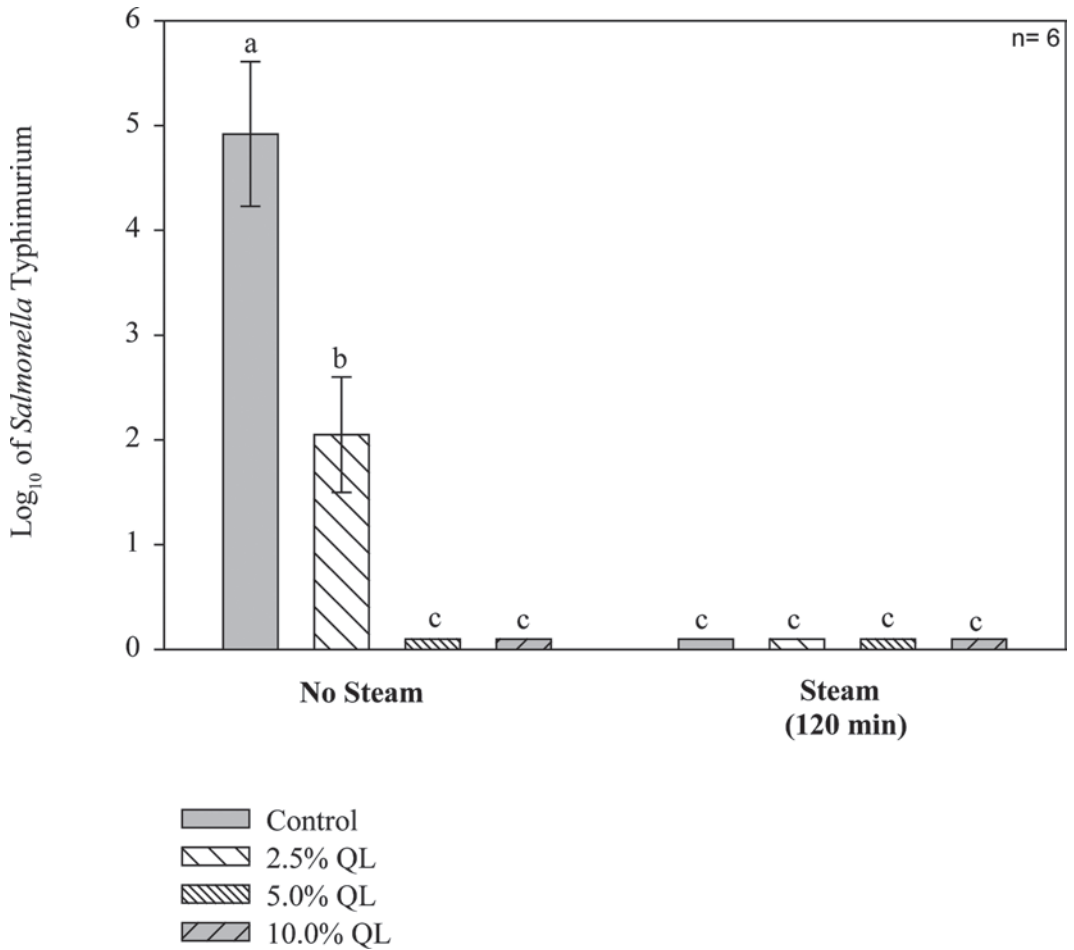


Figure 1. Evaluation of quicklime (QL) and 120 or 0 min of steam treatment on *Salmonella Typhimurium* recovery of used poultry litter. Means with different letters (a–c) differ significantly ($P \leq 0.05$).

izing poultry litter in a typical broiler house with steam. Placing a 4-in. (10.1-cm) layer of litter in a steam cart would not be practical for commercial purposes. However, insulated tarpaulins may be used to localize steam at the surface of the bedding material, as is done by commercial plant nurseries to pasteurize soil.

In the current study, production parameters were not evaluated after steam or QL treatment, or both. However, research has been conducted analyzing these parameters in poultry reared on litter treated with QL and hydrated lime. Bennett et al. [2] showed that hydrated lime in the litter at concentrations of 0.2, 1.0, or 5.0% increased BW in turkey poults and significantly reduced overall aerobic bacteria. Additionally, an increase in BW gain was observed at 7 wk

of age in turkeys reared in a pen treated with 0.2% hydrated lime. Ruiz et al. [31] showed that broilers reared for 42 d on 10 or 15% QL-treated litter did not develop any breast or footpad blisters, and that there were no negative effects on bird performance (BW, feed consumption, feed conversion, and mortality). Breast and footpad blisters, respiratory diseases, ocular irritation, and discomfort have all been directly correlated with increased ammonia levels in broiler houses [32]. Litter additives, such as monobasic calcium phosphate and phosphoric acid [33], have reduced litter pH, minimized ammonia volatilization, and limited microbial activity. It is likely that an increased pH level caused by a QL-steam treatment would also have a negative effect on microbial populations that generate

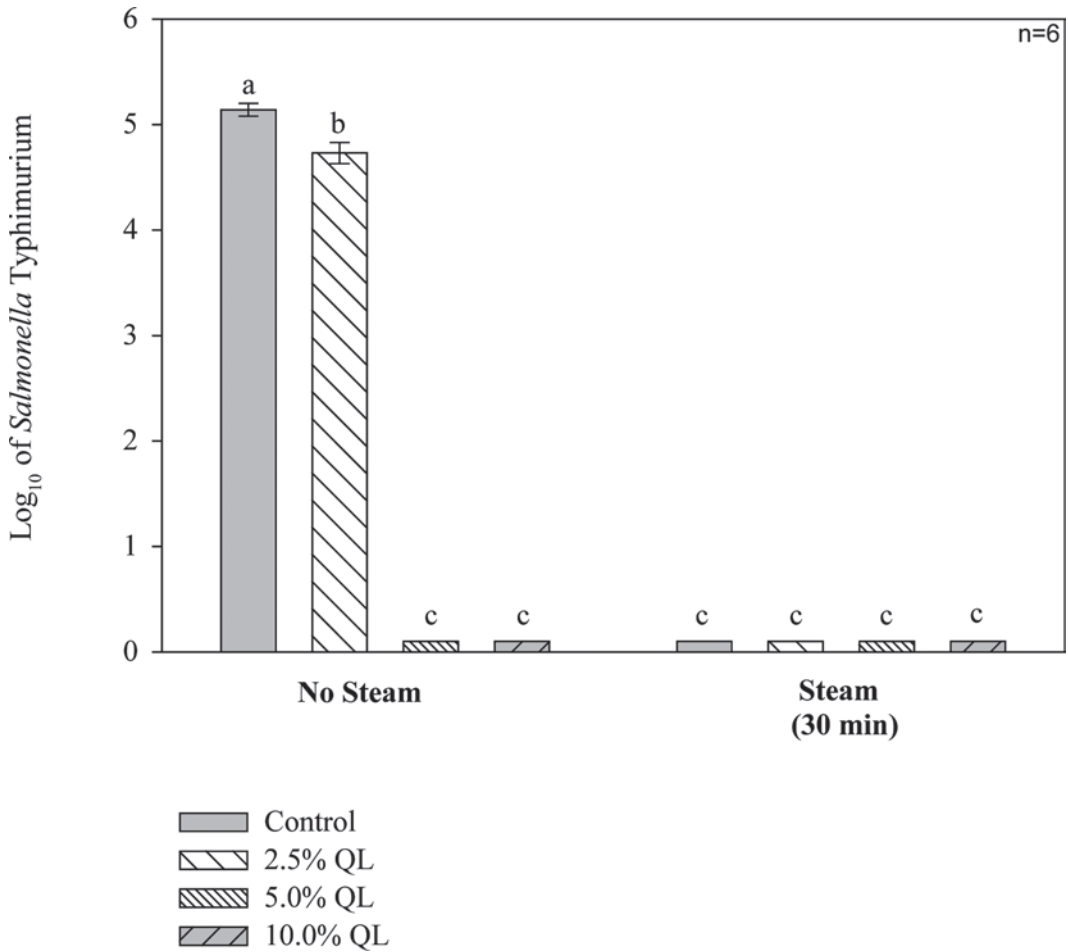


Figure 2. Evaluation of quicklime (QL) and 30 or 0 min of steam treatment on *Salmonella* Typhimurium recovery of used poultry litter. Means with different letters (a–c) differ significantly ($P \leq 0.05$).

ammonia gas that would be similar to the activity of these litter amendments [2]. However, elevated pH levels of the litter, as a result of a QL-steam treatment, will be unable to prevent the liberation of ammonia initially [34]. It has been speculated that reducing uric acid-splitting bacteria would eventually result in reduced generation of ammonia [2]. Additionally, high temperatures and low ventilation rates during the brooding period may cause complications as a result of increased ammonia volatilization, which may result in poor chick quality. Elevated temperatures accelerate bacterial ammonification, gaseous ammonia production, and mass transfer of ammonia from the litter to the rearing atmosphere [34].

To facilitate the exothermic reaction of QL, water needs to be added to the litter. Excess litter moisture and RH are also factors that contribute to the formation of ammonia [35]. In the current study, moisture content was determined after the addition of distilled H₂O and was typically 50 to 62% (data not shown). An increase of 30% (45 to 75%) RH has been shown to cause ammonia levels to increase and become more variable [36]. Increasing ventilation rates could counter this effect, but the increased rates may increase energy costs in a cool environment. Although moisture must be adequate to facilitate an exothermic reaction with QL, Ruiz et al. [31] found that moisture percentage was not affected in litter treated with 10 or 15% QL after a

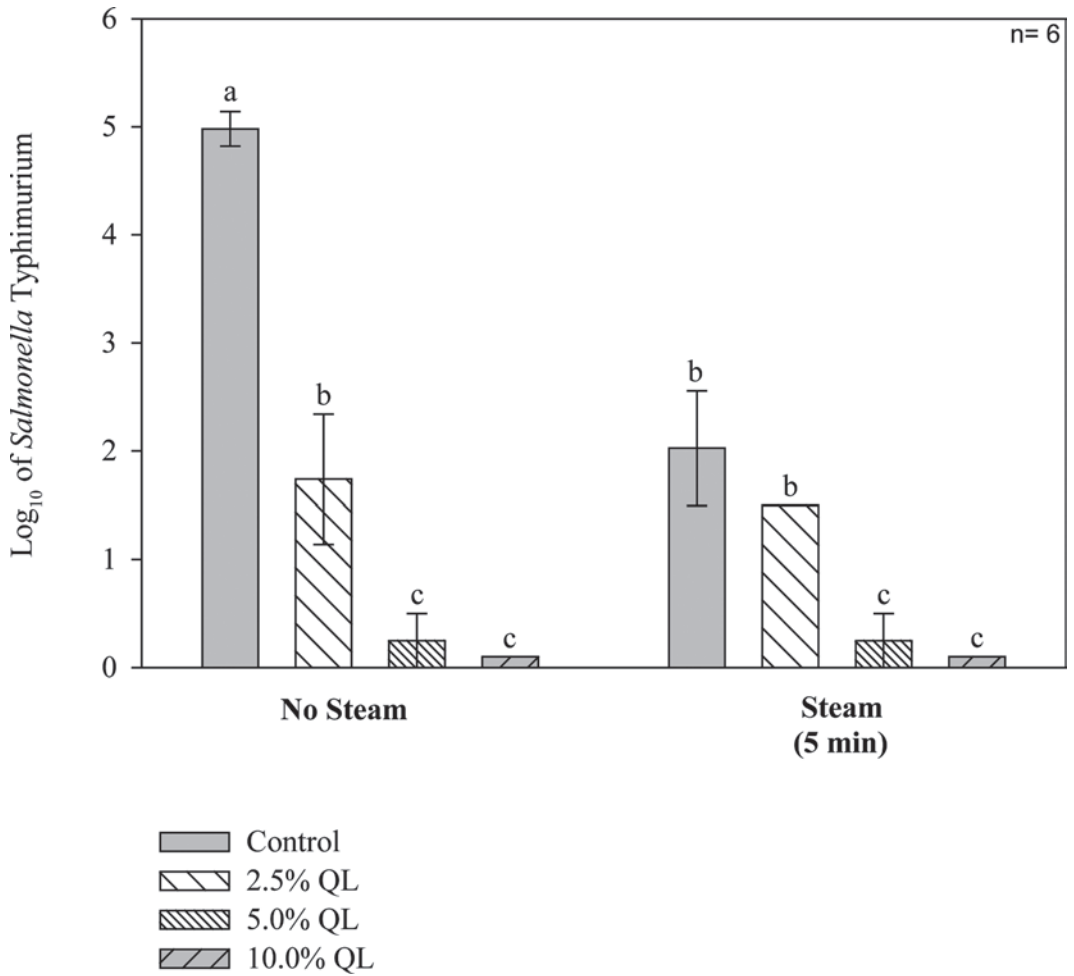


Figure 3. Evaluation of quicklime (QL) and 5 or 0 min of steam treatment on *Salmonella Typhimurium* recovery of used poultry litter. Means with different letters (a–c) differ significantly ($P \leq 0.05$).

42-d broiler grow-out. If the QL-treated litter is considered for land application, losses of phosphorus and pathogen runoff are of the greatest concern [10, 31]. Studies conducted by Maguire et al. [10] and Ruiz et al. [31] showed a 90% reduction of soluble phosphorus after lime treatment and found a reduction in nitrogen levels, possibly caused by ammonia volatilization.

Therefore, we conclude that steam pasteurization and QL may be an effective tool for reducing pathogen levels in poultry litter and improving litter quality for land application. Using steam to treat poultry litter is not currently practiced in the industry, but this may improve litter quality and bird health.

CONCLUSIONS AND APPLICATIONS

1. Steam and QL treatment of broiler litter inoculated with ST caused a significant reduction in bacteria, often reducing it to undetectable levels.
2. Quicklime alone caused a significant reduction in ST levels at 2.5, 5.0, and 10.0% compared with the untreated controls.
3. Steam alone caused a significant reduction in ST levels by at least 3 orders of magnitude compared with the untreated controls.

4. Further research is needed to determine the true effectiveness and practicality of litter pasteurization in a commercial poultry production facility.

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